Characterization of the Steady-State Folding Intermediates of Lysozyme with Fourier Transform Infrared Spectroscopy

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Using synchrotron FTIR, we will examine the secondary structure of lysozyme during the folding process on a sub-millisecond time scale. Lysozyme can be unfolded chemically and refolded by diluting out the denaturant. During folding, this enzyme displays very complex changes in secondary structure with time. With stopped flow UV-CD measurements three kinetic phases were detected. First, the burst phase occurred within the first two milliseconds, at which time the secondary structure, as measured by the UV-CD, reached a level similar to that of the native enzyme. Quite remarkably, the secondary structure continued to develop and at 80 ms reached a level 50% greater than that of the native enzyme! The ellipticity then returned to the native value with a time constant of about 300 ms. With the infrared measurements we expect to be able to follow this complete process but get more information about the specific folding motifs from the more sensitive infrared technique. The deconvolution of the Amide I band (1600 - 1700 cm^{-1}), provides details of the percent of the protein structure that is α-helical, β-sheet, β-turn, or extended coil. In our experiments, lysozyme is unfolded with pH and refolded by adding salt (KCl). Before examining the folding intermediates in a time-resolved fashion with the rapid-mixer first we are determining the equilibrium folding and unfolding conditions. We are combining circular dichroism, fluorescence, and infrared spectroscopies to confirm the endpoints of the reaction. It was further found that the deconvolution of Amide III band (1230 – 1100 cm-1) provides details of the percent of the protein structure, in similar fashion as that of Amide I band. Since this band occurs outside the absorption region of liquid water, the analysis of the folding intermediates need not be done in D2O solutions. Analysis of Amide I and Amide III bands give similar percentages for α-helix, β-turns, β-sheet and coil.